Automated On-line [11C]Methylation System

III. 1. Automated On-line [^{11}C]Methylation System

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Introduction

[^{11}C]Methylation by [^{11}C]methyl iodide is currently a powerful method for the rapid preparation of ^{11}C-labeled compounds, and due to the high specific activity many of them have been dedicated to brain receptor studies as positron-emitting neuroleptics. Their preparation can be achieved by rapid processing using HPLC purification after the [^{11}C]methylation. Some notable attempts have been made to automate the interfacing procedure between the [^{11}C]methylation and the purification by HPLC[1,2]. We have developed a new, general method readily adoptable for an automated system for the preparation of ^{11}C-radiopharmaceuticals using [^{11}C]methyl iodide.

Materials and Methods

Figure 1 shows a flow chart of the automated on-line [^{11}C]methylation system. The on-line [^{11}C]methylation procedure consists of the following steps 1): trapping of [^{11}C]methyl iodide by an appropriate adsorber, silica gel or Porapak Q, in a short column, replacing the sample loop of an HPLC injector (6-way valve) 2): loading a suitable solvent (usually DMF) into the column and heating for the reaction 3): injecting the reaction mixture into a HPLC column by switching the injector. The substrate is coated on an inert support (Flusin T), which is then mixed with the adsorber, and the .mixture is loaded into the column (internal volume: 55 μl).

The automated system developed is schematically illustrated in Fig. 2. It was constructed using pneumatically actuated devices such as valves and cylinders. A 6-way valve (Rheodyne Model 7000L switching valve with actuator) and two 3-way valves (Rheodyne Model 5301 3-way Slidervalve with Model 5300 actuator) were purchased from a commercial source and incorporated as the main parts of the system. A pneumatic syringe pump was specially designed to pull down a 2.5 ml disposable polypropylene syringe (Aldrich) containing a reaction solvent. The plate assembling the valves and the pump was attached to the vertical cylinder and also this part to the horizontal cylinder (CKD Unit Cylinder Model UCA-X/Y). All the cylinders were equipped with limit switches to send a signal to a controller at the completion of each movement.

A miniature programmable controller (PC; Izumi Denki Micro-1) was used as controller. The program sequence consists of 6 steps as follows:

Step 1. Check leaking in the system before starting the radiosynthesis.
Step 2. Dip the reaction column in an acetonitrile-dryice bath (-42°C) and then trap [^{11}C]methyl iodide.
Step 3. Inject 0.2 ml of a reaction solvent into the column.
Step 4. Move the reaction column from the cold bath to the oil bath regulated at 80°C to carry out the reaction for 5 min.
Step 5. Inject the whole reaction mixture into a semi-preparative HPLC column and keep injecting for 50 sec.
Step 6. Collect the product peak in a flask for solvent evaporation.

[^{11}C]Methyl iodide was prepared from [^{11}C]carbon dioxide using the fully automated synthesis system (NKK Co., Japan). When [^{11}C]carbon dioxide was introduced into the automated [^{11}C]methyl iodide system immediately after the irradiation, Step 2 was started and the accumulation of radioactivity in the reaction column was monitored with the radiation sensor. The next step was activated when the accumulated radioactivity was maximal (generally in less than 4 min), and the successive Steps 3 and 4 were carried out automatically. To determine radiochemical yields, all the radioactivity eluting from the HPLC column was collected in bottles and assayed for radioactivity measurement. After complete elution of the product the HPLC column was washed with 40 ml of ethanol and the radioactivity in the washings, as well as in the reaction and charcoal columns, was measured.

Results and discussion

Obvious advantages of the present system are 1) injection of a reaction mixture into a HPLC column without significant loss and 2) ready applicability to an automated system. Some typical results obtained for the present study are listed in Table 1. The optimal ratio of the adsorber to the support coated with the substrate was determined to be 35/20 (μl/μl) from the correlation curve between the trapping efficiency of [^{11}C]methyl iodide and the amount of silica gel as shown in Fig. 3. Figure 4 demonstrates that a higher radiochemical yield can be obtained by increasing the concentration of substrate. Under these conditions the reaction yields listed in Table 1 are apparently comparable to or even higher than those reported in the literature probably owing to another advantageous features of the present method, *i. e.* a high concentration of substrate in small portion of solvent can easily be attained and the reaction column has no vacancy inside to allow [^{11}C]methyl iodide to evaporate during the reaction.

The key idea of the present on-line [^{11}C]methylation is the incorporation of a short column, which plays the triple role of adsorber, reaction vessel and injection loop, in a HPLC injector. Watkins et al.[1] used a teflon loop as reaction column connected with a 4 way valve

which allowed only conventional liquid column chromatography for the following purification. In our system, by contrast, HPLC purification can be carried out by adapting an HPLC precolumn and HPLC injector. However, the latter method differs more fundamentally and essentially from the former in the method of trapping [^{11}C]methyl iodide. The on-line adsorption enables improvement of the radiosyntheses and adds more advantageous features to the system in terms of time, radiochemical yield, purification and amount of substrate as described above. Thus, the on-line [^{11}C]methylation has simplified the whole synthetic procedure and easily accommodated it to automation as demonstrated in the present study.

References

1) Watkins G. L., et al., Appl. Radiat. Isot. 39(1988)441.
2) Mulholland G. K., Jewett D. M. and Toorongian S. A., Appl. Radiat. Isot. 39(1988)373.

Table 1 On-line [^{11}C]Methylation of receptor ligands using [^{11}C]MEethyl iodide

Product	Method	Reaction conditions								Results				
		Column net vol. (μL)	Substrate Dose (mmol)	Conc. (mmol/mL)	Adsorber Matrl.	Vol. (mL)	Base conc. (mM)	Reaction Time (min)	Temp. (C)	Carrier amount (nmol)	Trapping efficiency (%)	Product yield On-column (%)	Overall (%)	Column residue (%)
BZT	A	200	1.5	17	SG	200	-	5.0	70	528	>99	38	38	
	A	55	6.1	68	SG	55	-	5.0	70	385	>99	86	86	6.3
DOX	A	55	6.0	71	SG	55	-	5.0	80	84	>99	69.5	69.5	12.9
	B	55	1.9	-	SG	47	-	5.0	80	317	>99	48.5	48.5	6.7
	B	22	1.5	-	SG	15	-	5.0	80	47	85.4	76.8	65.6	6.0
	B	55	3.8	-	SG	38	-	5.0	80	59	>99	71.5	71.5	7.1
	B	55	3.8	-	PQ	38	-	5.0	80	46	>99	60.2	60.2	11.8
	B	55	6.0	-	SG	29	-	5.0	80	28	98.2	83.0	81.5	4.1
CYH	B	55	3.5	-	SG	35	-	5.0	80	27	>99	86.9	86.9	4.2
	B	12.5	0.8	-	SG	8	-	5.0	80	88	75.2	79.7	59.5	7.2
NMS	C	55	3.5	-	PQ	35	25/Tw	5.0	80	47	>99	46.0	46.0	9.7
	C	55	3.5	-	PQ	35	25/Tw	5.0	80	21	>99	40.0	40.0	14.2
	C	55	3.5	-	PQ	35	25/Tm	5.0	80	29	>99	60.6	60.6	7.6

Product BZT: Benztropine, DOX: Doxepin, CYH: Cyproheptadine, NMS: N-methylspiperone
Method A: the substrate was loaded into a column with in DMF after trapping MeI by the adsorber.
B: the substrate coated on Flusin T was charged in a column with the adsorber in advance. DMF was loaded after MeI trapping.
C: the substrate coated on Flusin T was charged in a column with the adsorber in advance. DMF and TBAH were loaded after MeI trapping.
Adsorber SG: silica gel (80-100 mesh), PQ: Porapak Q (80-100 mesh).
Base Tw: Tetrabutyl ammonium hydroxide(TBA) in water, Tm: TBA in methanol.

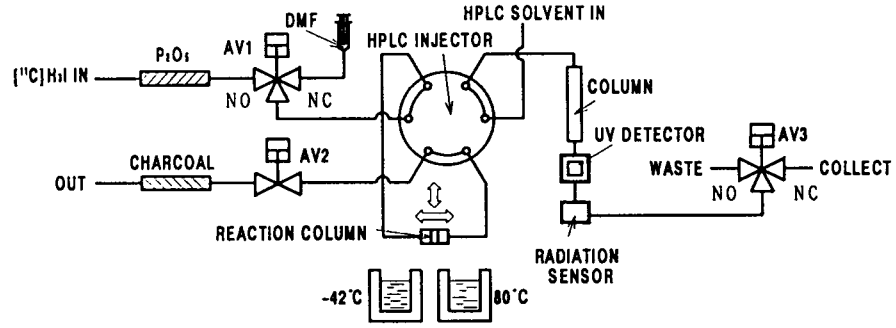

Fig. 1. A flow chart of the on-line [^{11}C]methylation system.

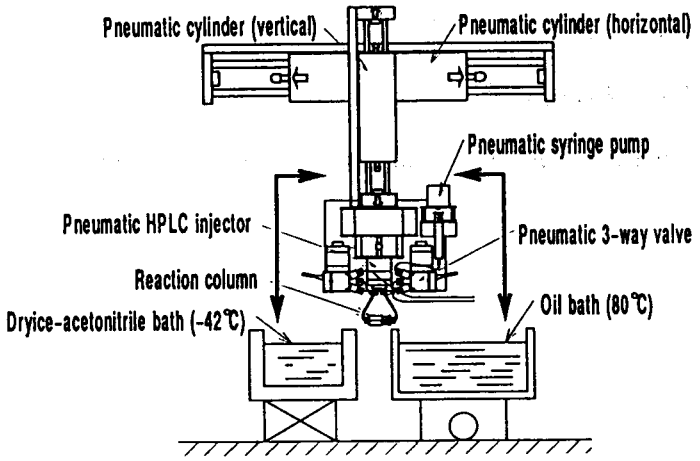

Fig. 2. A schematic diagram of the automated on-line [^{11}C]methylation system.

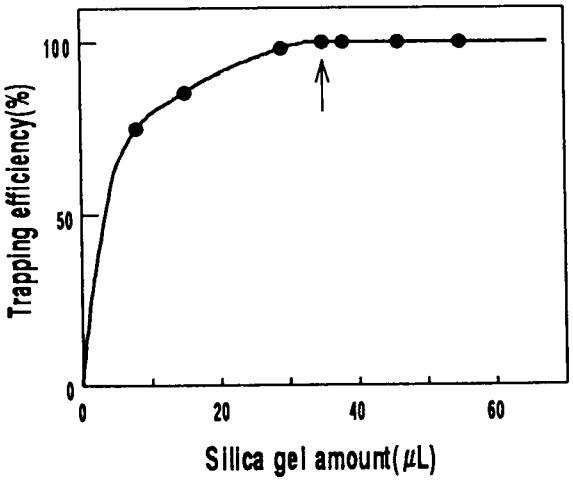

Fig. 3. Correlation between the amount of silica gel and the trapping efficiency of [^{11}C]methyl iodide.

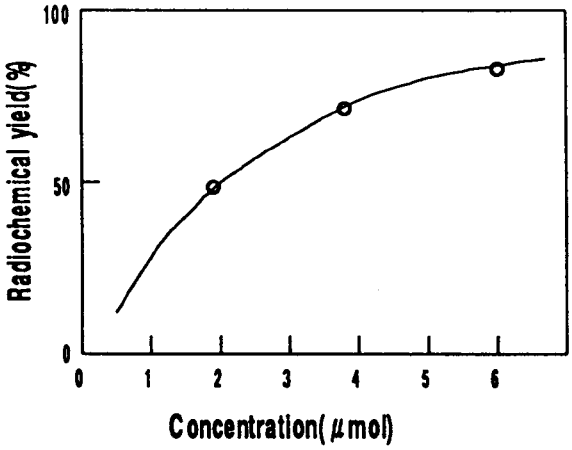

Fig. 4. Effect of the substrate concentration on the radiochemical yield in the [^{11}C]doxepin synthesis.